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14. ABSTRACT

Background: Combination of the cytotoxic viral thymidine kinase (tk) and the prodrug, acyclovir (ACV) has been reported to inhibit the growth of the C4-2 tumor, a subline of LNCaP. However, it remains unsolved to non-invasively detect the in vivo distribution, expression and persistence of the toxic gene as well as to evaluate the therapeutic effect. In this project, we will develop a nuclear gene imaging approach to assist the cytotoxic gene therapy study for prostate cancer.
Objective/Hypothesis: The distribution, expression, and persistence of the prostate specific Ad-PSA-tk in the C4-2 tumor xenograft model will be non-invasively and repeatedly determined in vivo by tracing the radiolabeled TK substrates with a SPECT imaging modality.
Specific Aim of the first year: To synthesize a radiolabeled TK substrate, 2'-Deoxy-2'fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine, for TK detection using a small animal gamma detector.
Progress and outcome: In last report of 2003 which covers from September of 2002 to March of 2003, we reported our efforts to synthesize fragments A and B. In this report we successfully linked the radiometal chelator with fluorothymidine. We will characterize the structure of the final tracer and test the pharmacokinetics and pharmacodynamics of the tracer in next research year. Also, the Adenoviral vectors with reporter genes of tk and luciferase were constructed. The luciferase gene expression in live mouse model was non-invasively imaged and the result was posted in 2003 Annual Meeting of ASGT (American Society of Gene Therapy).

15. SUBJECT TERMS
prostate cancer, gene therapy, imaging

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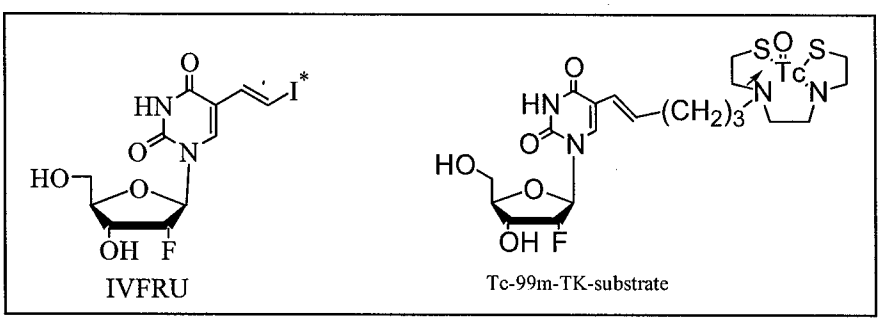
Introduction

The objective of this project is to develop a noninvasive imaging assay using single photon emission computed tomography (SPECT) for assessment of gene therapeutic efficacy and diagnosis of metastasis of prostate cancer.

Currently, nuclear imaging technology has demonstrated the greatest potential to non-invasively image gene activity in animals and humans due to its high sensitivity. By replacing the acyclovir (ACV) with a radioactive analogue, it is possible to non-invasively and repeatedly monitor the *in vivo* distribution of the transduced tk construct. It may assist in determining the optimal timing for ACV administration, confirming the cytotoxic sites, and assessing the therapeutic efficacy. Further refinement of this technology could also provide a non-invasive approach to identify any metastasis sites in a clinical setting.



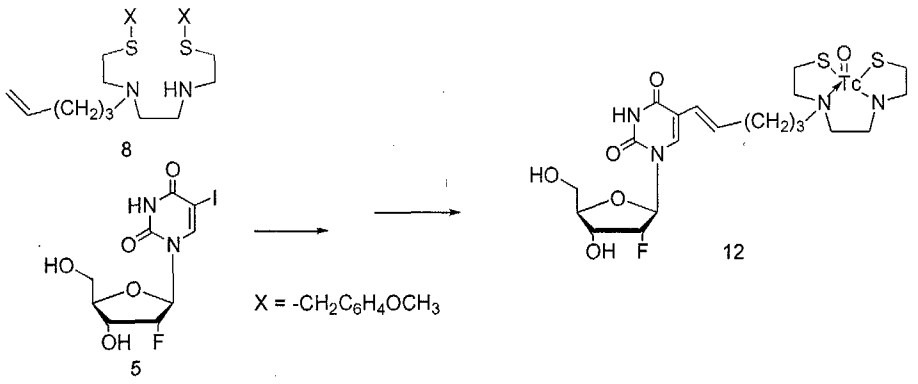
In the original plan, we proposed to synthesize a novel thymidine kinase (TK) substrate, I-123 labeled 1-(2-deoxy-2-fluoro-β-D-ribofuranosyl)-5(E)-(2-iodovinyl)uracil (IVFRU). However, the recent progress of Tc-99m chemistry that Tc-99m labeled radiopharmaceuticals, such as TRODAT, being able to penetrate lipid membrane raises our interest to synthesize a Tc-99m labeled TK substrate for gene imaging, because of the nearly optimal nuclear properties of Tc-99m, as well as its convenient and low cost production by means of commercial generator columns. As a result, we modified our plan by switching the target molecule, [I-123]IVFRU with 2'-Deoxy-2'fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine.

In biological experiment, we constructed prostate specific adenovirus vector, Ad-PSA-TK. To test the target specificity of PSA promoter, viral vector Ad-PSA-Luc was constructed and a charge coupled device (CCD) video camera was used to image noninvasively human prostate tumors and metastases in nude mice after injection of $2x10^9$ PFU of Ad-PSA-Luc virus via tail vein.

Body

1. *Chemistry*

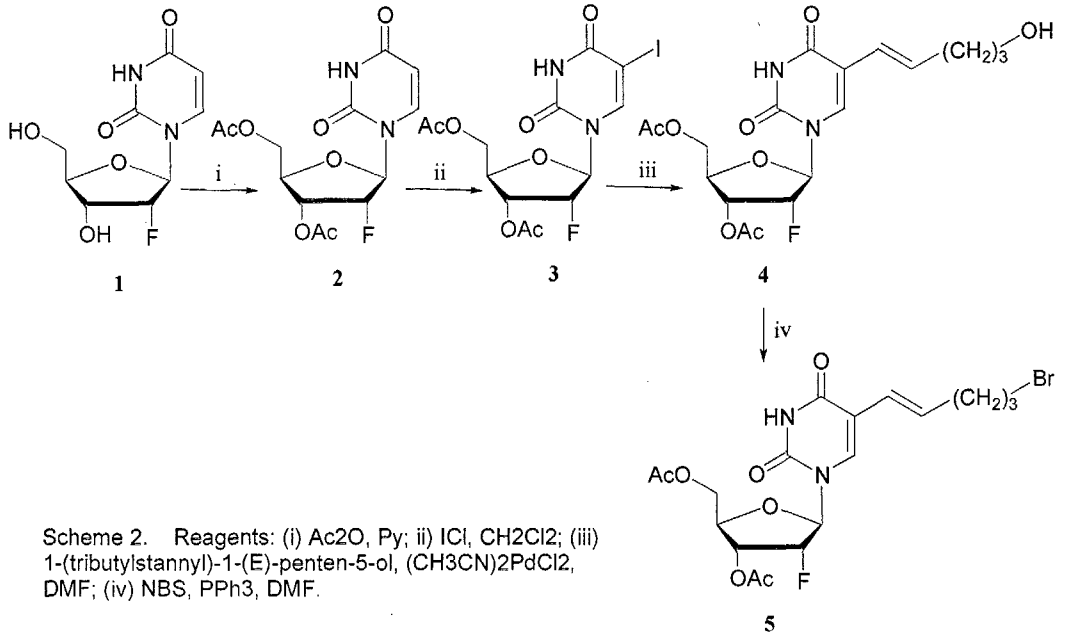
The target molecule, 2'-Deoxy-2'fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine **12**, was convergently synthesized from synthons **5** and **8**. The detail of the synthesis was reported in *Tetrahedron Letters*, 2004, 45, 8673-8676.



8 5 12

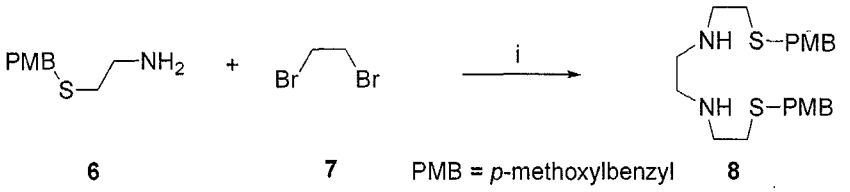
Scheme 1

Briefly, the synthesis of 5-(5-bromopent-1(*E*)-enyl)-1-(3,5-diacetyl-2-fluoro-2-deoxy-1-β-D-ribofuranosyl)uracil **5** and *N*,*N*′-bis-[2-(4-methoxybenzylsulfanyl)ethyl]ethylenediamine **8** are outlined in scheme 2 and 3.



1 2 3 4 5

Scheme 2. Reagents: (i) Ac2O, Py; ii) ICl, CH2Cl2; (iii) 1-(tributylstannyl)-1-(E)-penten-5-ol, (CH3CN)2PdCl2, DMF; (iv) NBS, PPh3, DMF.

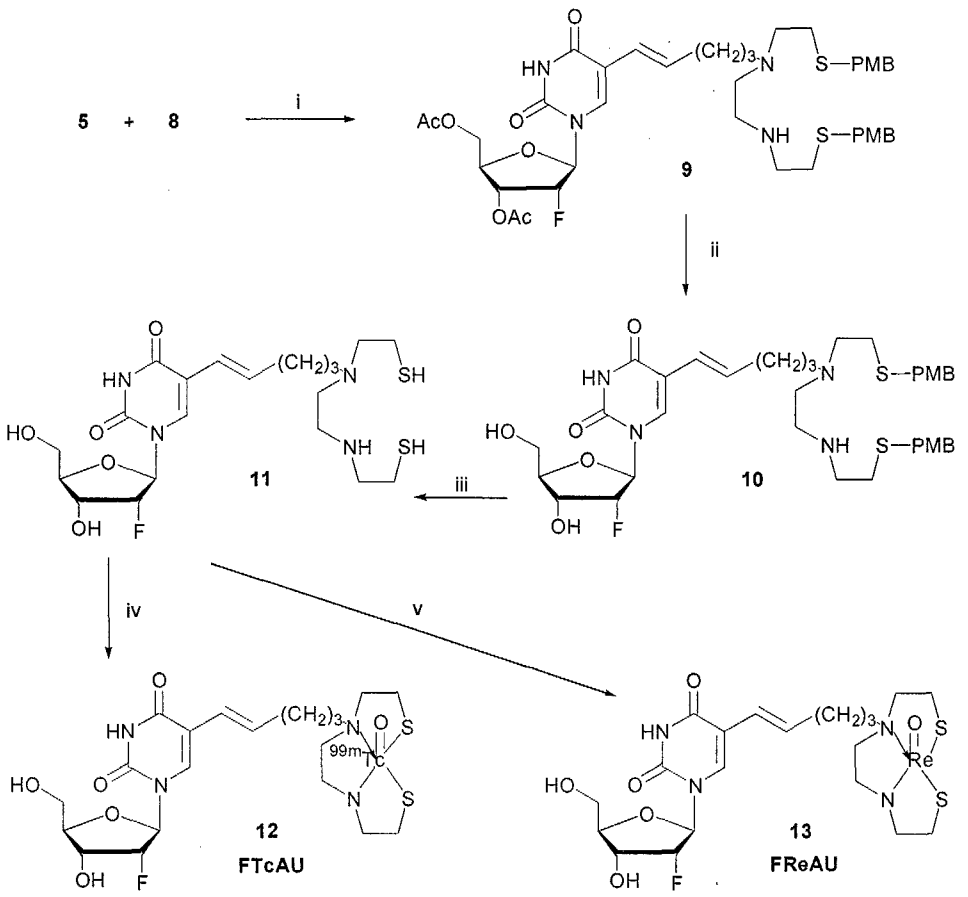


6 7 8

Scheme 3. Reagents: (i) CeOH, molecular sieve (4Å), DMF.

Coupling of thymidine analog **5** and chelator fragment **8** produces **9**. The removal of acetyl protecting groups of **9** with potassium carbonate in aqueous methanol yields **10**.

The thiol protecting groups, 4-methoxybenzyl, of **10** are removed with $Hg(OAc)_2$ in TFA to give trifluoroacetate salts of **11**. The crude air-sensitive compound **11** is conjugated with technetium immediately, without purification. Addition of [^{99m}Tc]pertechnetate in PBS into the aqueous methanol of the crude **11** in the presence of Sn-glucoheptonate in 80°C water bath for 30 min and thereafter HPLC purification yields the target compound, FTcAU **12** with radiochemical yield of 42%. To characterize the chemical structure of the FTcAU **12**, its analog of rhenium-188 conjugate, FReAU **13**, is synthesized with modifying a similar reaction condition by adding tetrabutylammonium tetrachlorooxorhenate(V) into a solution of compound **11** in methanol and stirring for 12 hours. The rhenium conjugate **13** is purified by flash chromotography and its chemical structure is characterized with ^{1}HNMR and high resolution ESI-MS. The characterization of FTcAU is carried out using reverse phase HPLC by co-injection with FReAU (schem 4).



Reagents: (i) DIEA, CH3CN; (ii) K2CO3; (iii) Hg(OAc)2/TFA, H2S; (iv) [99mTc]NaTcO4, Sn-gluceptate (v) (Bu4N)+(ReOCl4)-.

Scheme 4

2. *Biology*

In summary, we demonstrated that the AdPSA-Luc can generate high level expression of luciferase gene under the control of the 5837 bp long PSA promoter in

lungs of normal mice via tail vein injection. To our knowledge, this is the first report that unequivocally demonstrates specific gene expression in lung tissue elicited by a PSA promoter. This may predict PSA expression in lungs of normal mice. These results indicate the potential limitations of the suicide gene therapy of prostate cancer based on the selectivity of PSA promoter. By contrary, it has encouraging implications for the further development of vectors via PSA to enable gene therapy for pulmonary vascular diseases.Viral Vector

Key Research Accomplishments:

Chemistry: The Tc-99m labeled potential imaging tracer, 2'-Deoxy-2'fluoro-5-{3-oxo[N,N-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(E)-propenyl}uridine, was successfully synthesized.

Biology: Normal lung tissue of nude mice demonstrates specific gene expression elicited by a PSA promoter.

Reportable Outcomes:

Chemistry: Synthesis of a potential Tc-99m labeled TK substrate was published in *Tetrahedron Letters*;

Biology: Highly specific expression of luciferase gene in lungs of naïve nude mice directed by prostate-specific antigen promoter was reported in *Biochemical and Biophysical Research Communications.*

Conclusions:

1. We have obtained a Tc-99m labeled thymidine analog and evaluation of its activity as TK substrate is undergoing.
2. Highly specific gene expression in lung tissue elicited by a PSA promoter predicts PSA expression in lungs of normal mice.

Reference:

1. Synthesis of a novel Tc-99m labeled TK repot probe, 2'-Deoxy-2'fluoro-5-{3-oxo[*N,N*-bis(2-mercaptoethyl)ethylenediaminato][Tc-99m] technetium(V)-1(*E*)-propenyl}uridine, Y, Zhang, X. Dai, D. Kallmes and D. Pan, *Tetrahedron Letters*, 2004, 45, 8673-8676.
2. Highly specific expression of luciferase gene in lungs of naïve nude mice directed by prostate-specific antigen promoter, H. Li, J. Li, G. Helm and D. Pan, *Biochemical and Biophysical Research Communications*, 2005, 334(4), 1287-1291.
3. Highly Specific Expression of the Luciferase Gene in Lungs of Naïve Nude Mice Directed by Prostate Specific Antigen Promoter, Hongwei Li, Jin Zhong Li, Gregory A. Helm, Dongfeng Pan, *The 8th Annual Meeting of the American Society of Gene Therapy,* June 1-5, 2005, St. Louis, MO.